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Incorporation of 5-Fluorouracil Into Hepatoma and Normal Tissue RNA at Protein Depletion in the Rat

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Background and Objectives: 5-fluorouracil (5-FU) exerts its effects mainly by its incorporation into RNA and inhibition of DNA synthesis. Its toxicity may therefore be estimated by measuring its incorporation into RNA. Protein malnutrition has been considered to increase the toxicity of 5-FU.
Methods: Rats with a hepatoma implanted into the liver were fed on either a 25% or a 0% casein diet for 1 week. On the last day, they were infused via the hepatic artery with a therapeutic dose of ^{3}H-5-FU. Its incorporation into RNA was measured in hepatoma and several normal tissues.
Results: Protein deprivation increased the incorporation of 5-FU into liver and intestinal RNA. Incorporation into hepatoma RNA did not increase significantly, but the ratio, liver/hepatoma RNA incorporation, remained unchanged.
Conclusions: Protein deprivation might increase the toxicity of 5-FU on liver and intestine. *J. Surg. Oncol. 1997;65:155–158.*

KEY WORDS: liver; intestine; kidney; protein malnutrition; rat; DNA

INTRODUCTION

5-FU exerts its effects mainly by inhibiting DNA synthesis and by its incorporation into RNA and to some extent into DNA. In several tumor systems the cytotoxicity of 5-fluoropyrimidines appeared to be related to their incorporation into RNA [1]. Their toxicity to normal tissues, especially those with low DNA synthesis as the liver, may therefore be estimated by measuring its incorporation into RNA. Incorporation into the acid soluble fraction (ASF) and DNA is measured in the same analysis. Protein malnutrition is of special interest in this context, as it is well known to alter nucleic acid synthesis in several tissues and to increase the incorporation of precursors into liver RNA [2,3]. In addition, tumor-bearing mice fed on a high protein diet and treated with 5-FU survived longer than mice fed on a low protein diet [4]. As a part of investigations on the modulation of 5-FU anabolism, we therefore measured its incorporation into ASF, RNA, and DNA of liver, small intestine, kidney, spleen, bone marrow, and a hepatoma, inoculated into the liver in protein-depleted rats. Hepatoma was chosen to enable comparison with normal liver. The liver nucleotide profile was analyzed with isotachophoresis.

MATERIALS AND METHODS

Animals and Tumor

Listar Hooded rats (B&K Universal, Sollentuna, Sweden) were fed a standard laboratory diet (Ewos R3; Lactamin AB, Stockholm, Sweden) and tap water ad libitum prior to the experiment. The tumor was a 3′-methyldiaminobenzidine induced hepatoma, delivered by the Department of Surgery, University of Gothenburg, Sweden, maintained by intramuscular passage. The rats were kept in filter-top cages until the experiments were performed. Generations 527 and 530 were used. The ultrastructure of the hepatoma has been described [5]. It does not contain peroxisomes. The experiment was approved by the Malmö-Lund Ethical Board for animal experiments.

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Experimental Design

Through a midline incision, a suspension containing 1.0×10^6 vital tumor cells was inoculated under direct vision into the left lateral (''central'') liver lobe. Eight days later (= 2 days before killing), a catheter (PE 10, Portex, Hythe, Kent, UK) with an inner and outer diameter of 0.28 and 0.61 mm, respectively, was tapered and inserted retrogradely into the gastroduodenal artery with the tip close to the origin of the proper hepatic artery at laparotomy, tunnelled subcutaneously, and brought out in the distal part of the tail. The rats were placed individually in restraining cages with the catheters connected to an infusion pump (type 1850, Braun AG, Melsungen, Germany), delivering 1 ml 0.9% NaCl solution per 24 hr containing 10 I.U. heparin/ml. The animals were fed ad libitum on either a 25% casein or a nonprotein diet [6] and tap water the last 7 days before killing. The body weights were 216 (193–253) g (median, range) and 218 (201–248) g, respectively, on the day the rats were put on these diets.

Two days after catheter insertion the rats were infused via the hepatic artery over 2 hr with 1,200 nmole 5-FU + 0.25 mCi 5-[6-^{3}H]-FU (15.0 Ci/mmol, New England Nuclear, Boston, MA) in a volume of 1 ml, a dose suitable for experiments on uptake and distribution of 5-FU in tissues of rats weighing ~200 g [7]. All rats were killed 1 hr after the end of the infusion around noon.

For the isotachophoretic analysis of nucleotides, one piece of the median liver lobe was freeze-clamped in situ under anaesthesia between a pair of tongs precooled in liquid nitrogen. The tumor, most of the remainder of the liver, left kidney, spleen, and a piece of the terminal ileum, which was washed with cold saline, and femoral bone marrow were removed, immersed in liquid nitrogen, and stored at −70°C. The chemical analysis was performed as described previously [7].

Isotachophoresis is an electrophoretic separation technique using a capillary tube with a leading and a terminating electrolyte, the latter with a lower effective mobility. The sample ions are injected in between. When an electric current is applied, the sample ions will migrate with different velocities due to their different effective mobilities, but will then arrange in zones according to these differences and move in strict order and with the same velocities. The zones, in our system the different nucleotides, can be measured by their ultraviolet absorbance. Nonabsorbing spacers are added to the samples to separate the individual nucleotides. The fluoronucleotides comigrate with their normal counterparts and are therefore reported as (F)UTP etc [8,9].

Because it was impossible to weigh the rats before 5-FU administration due to the fixed catheters, all animals were given the same amount of 5-FU and weighed at sacrifice. The results were corrected, with consideration of the final body weight, to make the injections equivalent. The casein-fed rats decreased 8 g (0– −17) in weight, the rats on the protein-free diet 29 g (−26– −39) during the experiment ($P < 0.001$).

Anaesthesia

Tumor inoculation was performed under chloral hydrate anaesthesia (50 mg/ml; 0.75 ml/100 g body weight intraperitoneally). Insertion of catheter and killing of rats were performed under isoflurane anaesthesia. Rats were given a gaseous mixture of 1 l/min oxygen, 2 l/min nitrous oxide (N_2O), and 4% isoflurane for 3 min as induction and then maintained on 1.5% isoflurane.

Statistical Analysis

The Mann-Whitney U-test was used for statistical comparisons. All tests were two-sided and *P*-values of <0.05 were considered statistically significant.

RESULTS

In the rats fed the noncasein diet, there was a decrease in the RNA/DNA ratio in the liver and an increase in the incorporation of 5-FU into RNA (Table I). In the small intestine, the incorporation into ASF, RNA and DNA increased.

The renal nucleotide (NT)/DNA and RNA/DNA ratios decreased at protein deprivation with a concomitant increase in the mg DNA/g tissue ratio. The incorporation of 5-FU into the low molecular acid soluble fraction increased but not into the high molecular weight RNA.

In the spleen, the 5-FU incorporation into DNA decreased at protein deprivation. The RNA/DNA ratio decreased. In the bone marrow, the only change was a slight increase in the incorporation of 5-FU into the acid soluble fraction in protein deprivation.

The median value for incorporation of 5-FU into tumor RNA increased at protein depletion. The range was large and there was no statistical difference. The ratio, liver/tumor RNA incorporation, was, however, the same in both groups ($P = 0.8$).

The liver nucleotide profile showed a lower level for several nucleotides at protein deprivation, tallying with the lower RNA contents per cell. Energy charge was, however, not decreased. There was no tendency to decrease (F)UDP-glucuronic acid (Table II).

The weight of the tumors were the same in both groups: 0.48 g (0.28–1.95) in protein-fed, 0.44 g (0.14–0.82) in protein-deprived rats ($P = 0.7$).

DISCUSSION

The highest incorporation of 5-FU into RNA was obtained in liver and hepatoma, confirming the advantage of the hepatic arterial route for treatment of liver malignancies with 5-FU. There was an increased incorporation of 5-FU into liver RNA at protein deprivation. This may

have several explanations. First the liver nucleotide pool, calculated per cell, i.e., per mg DNA decreased at protein deprivation (Table I and ref. 2). Second, the de novo pyrimidine synthesis may be decreased [10]. Therefore, flooding the nucleotide pool with an RNA precursor as 5-FU may promote its incorporation into RNA. Furthermore, the ribosomal RNA synthesis appears to be increased in protein deprivation as suggested by increase in polymerase I activity [11,12] and increase in nucleolar size [5]. Normal labelled precursors such as orotic acid and cytidine [2,3] also show enhanced incorporation into liver RNA at protein deprivation. In addition, the activity of the 5-FU-degrading enzyme, dihydropyrimidine dehydrogenase, is decreased in protein deprivation [13]. Also, other enzymes of possible importance for 5-FU catabolism and excretion might be decreased.

Incorporation into hepatoma RNA was not statistically enhanced at protein deprivation. The median value for 5-FU incorporation into RNA increased, however, so that the ratio, liver/tumor RNA incorporation, was essentially unchanged (Table I). The same finding was obtained with a colon carcinoma [14]. The great variation in the tumor values, certainly due to the necrosis always present in the centres of solid tumors, appears to have prohibited possible significances to be found. It cannot therefore be excluded that feeding a protein-free diet also affects the sensitivity of a tumor to 5-FU. In addition, several studies indicate that a protein deficient diet may retard tumor growth [15–17].

The incorporation of 5-FU into intestinal RNA also increased at protein deprivation arguing for increased toxicity. That might tally with an increased incidence and duration of diarrhea in protein-depleted mice treated with 5-FU [18].

Protein malnutrition decreases the cellularity of the bone marrow [3,19], which to some degree parallels the decrease in body weight [19] but without increased 5-FU toxicity. On refeeding protein, when cellular proliferation resumes in blood-forming tissues, the 5-FU toxicity is temporarily increased [19]. Our findings of decreased incorporation of 5-FU into lienal DNA might reflect decreased white cell production during protein deprivation.

CONCLUSIONS

Highest incorporation of 5-FU into RNA was obtained in tumor and liver, which is no surprise with administration via the hepatic artery. The increased incorporation of 5-FU into hepatic and intestinal RNA at protein deprivation suggests an increased toxicity to these tissues.

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TABLE I. Amount (in μg) of Nucleotides (NT) and RNA (per μg) DNA and Incorporation of 5-fluorouracil Into mg Acid Soluble Fraction (ASF), RNA, and DNA, and Into ASF and RNA per mg DNA and mg DNA/g Tissue†

		μg/μg DNA		Picomole 5-FU into					mg DNA
Tissue	Diet	NT	RNA	ASF	ASF/DNA	RNA	RNA/DNA	DNA	g tissue
Tumor	Casein	0.24 (0.08–0.33)	0.66 (0.38–1.17)	5869 (1899–11906)	1057 (458–1601)	21 (2–192)	11 (1–169)	0.3 (0.1–1.8)	5.9 (4.1–6.4)
	Noncasein	0.31 (0.09–0.38)	1.02 (0.34–1.28)	4751 (3126–11465)	1186 (1011–2812)	45 (5–77)	34 (2–86)	0.4 (0.1–0.9)	5.8 (5.2–6.2)
Liver	Casein	1.54 (1.32–1.85)	4.28 (3.83–4.90)**	3248 (2730–6413)	5406 (3594–9152)	8.9 (6.8–16.8)**	37 (26–82)****	2.7 (1.9–2.9)	2.0 (1.5–2.3)
	Noncasein	1.36 (1.18–1.51)	3.57 (3.29–3.92)	3845 (2830–4203)	5056 (3724–6316)	21.4 (12.0–151)	80 (51–497)	2.6 (2.4–7.8)	2.2 (2.0–2.4)
Small	Casein	0.39 (0.35–0.42)	0.89 (0.80–1.01)	1384 (1227–1642)****	562 (486–609)	9.5 (4.8–15.2)****	8.3 (6.5–13.7)****	0.7 (0.3–2.0)***	6.2 (5.5–7.1)
intestine	Noncasein	0.36 (0.34–0.37)	0.82 (0.79–0.92)	1823 (1244–2434)	646 (438–855)	20.3 (8.0–36)	16.8 (6.7–29.4)	2.4 (0.8–4.8)	6.3 (5.5–6.7)
Kidney	Casein	0.74 (0.64–0.81)*	1.09 (1.01–1.20)*	4493 (3674–6024)**	3370 (2845–4216)****	10.2 (8.7–15.0)	11.4 (8.9–17.1)	0.7 (0.5–0.8)	3.8 (3.5–4.1)*
	Noncasein	0.60 (0.54–0.62)	0.91 (0.91–0.98)	6902 (4916–7618)	4144 (2957–4553)	12.6 (11.0–15.0)	11.2 (10.0–13.7)	0.7 (0.5–0.9)	4.4 (4.3–4.7)
Spleen	Casein	0.22 (0.21–0.27)	0.56 (0.52–0.68)***	2102 (1756–2363)	473 (402–568)	11.0 (6.3–53)	5.9 (3.2–36)	0.5 (0.3–0.8)**	11.9 (9.7–13.8)
	Noncasein	0.22 (0.20–0.23)	0.52 (0.48–0.54)	2250 (2093–2393)	476 (421–512)	12.7 (8.9–98)	6.6 (4.3–49)	0.3 (0.2–0.7)	12.5 (11.8–13.6)
Bone	Casein	0.15 (0.13–0.19)	0.50 (0.45–0.64)	1851 (1678–1955)**	285 (240–328)	12.8 (8.1–28)	6.0 (4.1–16.6)	0.9 (0.6–1.1)	18.6 (14.9–20.2)
marrow	Noncasein	0.15 (0.13–0.17)	0.48 (0.44–0.52)	1962 (1916–2123)	296 (270–328)	15.6 (11.0–22)	7.8 (5.0–10.3)	0.8 (0.5–1.3)	17.6 (16.3–20.0)

†There were 8 rats in each group. Values are medians (range).
Mann-Whitney's U-test. *$P < 0.001$; **$0.001 < P < 0.01$; ***$0.01 < P < 0.02$; ****$0.02 < P < 0.05$.

TABLE II. Amount in Liver of (F)UTP, GTP, (F)UDP-Glucuronic Acid (=(F)UDP-Glu), ATP, ADP, AMP, NAD, and Sum of Adenine Nucleotides (ΣATP, ADP, AMP) in Nmole/mg DNA, Energy Charge†

Diet	(F)UTP[a]	GTP[b]	(F)UDP-Glu[c]	ATP[d]	ADP[e]	AMP[f]	Σ(ATP,ADP,AMP)[g]	NAD[h]	Energy charge
Casein	173 (157–225)	226 (182–280)***	143 (100–211)	1333 (1052–1730)	532 (359–649)*	134 (102–189)*	2044 (1653–2545)**	394 (309–453)***	0.80 (0.77–0.83)
Noncasein	139 (118–204)	187 (166–242)	145 (123–196)	1096 (1019–1459)	358 (337–417)	98 (85–104)	1572 (1457–1886)	336 (292–391)	0.82 (0.80–0.85)

†There were 8 rats in each group. (F) signifies that the fluoro-compound and its normal counterpart are found in the same peak. Values are medians (range).
[a](fluoro) uridine triphosphate.
[b]Guanosine triphosphate.
[c](fluoro) uridine diphosphate glucuronic acid.
[d]Adenosine triphosphate.
[e]Adenosine diphosphate.
[f]Adenosine monophosphate.
[g]the sum of ATP, ADP, and AMP.
[h]Nicotinamide adenine dinucleotide.
Mann-Whitney's U-test: *$0.001<P<0.01$; **$0.01<P<0.02$; ***$0.02<P<0.05$.

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